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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

BIOASSAY OF

2,4-DIMETHOXYANILINE HYDROCHLORIDE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
U.S. National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

Carcinogenesis Technical report series

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REPORT ON THE BIOASSAY OF 2,4-DIMETHOXYANILINE HYDROCHLORIDE FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 2,4-dimethoxyaniline hydrochloride conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 2,4-dimethoxyaniline hydrochloride was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly. Chemical analysis was performed by Midwest Research Institute (6) and the analytical results were reviewed by Dr. N. Zimmerman (7).

Histopathologic examinations were performed by Dr. B. C. Zook (4), at Litton Bionetics, Inc., the pathology narratives were written by Dr. B. C. Zook (4), and the diagnoses included in this report represent the interpretation of this pathologist. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (8).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (9); the statistical analysis was performed by Mr. R. M. Helfand (7) and Dr. J. P. Dirkse, III (10) using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (11).

This report was prepared at METREK, a Division of The MITRE Corporation (7) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (7), task leader Ms. P. Walker (7), senior biologist Mr. M. Morse (7), biochemist Mr. S. C. Drill (7), and technical editor Ms. P. A. Miller (7). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,12), Dr. R. A. Griesemer (1), Dr. T. E. Hamm (1), Dr. W. V. Hartwell (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,13), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay for the possible carcinogenicity of 2,4-dimethoxyaniline HCl was conducted using Fischer 344 rats and B6C3F1 mice. 2,4-Dimethoxyaniline HCl was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 2,4-dimethoxyaniline HCl were, respectively, 3000 and 1500 ppm for rats and 5000 and 2500 ppm for mice. The compound was administered in the diet for 104 weeks to rats and 103 weeks to mice, followed by a 1-week observation period for both species.

There were no significant positive associations between the concentrations of 2,4-dimethoxyaniline HCl administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Dose-related mean body weight depression was observed for females of both species, indicating that the concentrations of 2,4-dimethoxyaniline HCl administered to these groups may have approximated the maximum tolerated concentrations. Compound-related mean body weight depression was only slight for male rats and was apparent in male mice only until week 50; however, follicular-cell hyperplasias and cystic follicles of the thyroid were observed in dosed male mice, suggesting that the concentrations the male mice received may have approximated the maximum tolerated concentrations. Since no distinct mean body weight depression in relation to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of 2,4-dimethoxyaniline HCl to male rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

There was a significant positive trend between concentration of the test chemical and the incidence of a combination of hepatocellular carcinomas and adenomas in male mice and an increase in the combination of these lesions in female mice. However, no statistically significant differences in tumor incidence at any specific site were observed when dosed rats and mice were compared to their respective controls.

Under the conditions of this bioassay there was no convincing evidence for the carcinogenicity of 2,4-dimethoxyaniline HCl in Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

2,4-Dimethoxyaniline hydrochloride (Figure 1) (NCI No. C02255), the hydrochloride salt of the dye intermediate 2,4-dimethoxyaniline, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer observed among dye manufacturing industry workers (Anthony and Thomas, 1970; Wynder et al., 1963). Aromatic amines are one of several classes of chemicals thought to contribute to the increased cancer risk in this industry (Clayson and Garner, 1976).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 2,4-dimethoxybenzenamine hydrochloride.* It is also called 4-methoxy-o-anisidine hydrochloride and 2-methoxy-p-anisidine hydrochloride.

2,4-Dimethoxyaniline hydrochloride does not appear to have any commercially significant applications. The major commercial use of 2,4-dimethoxyaniline is apparently as an intermediate in the production of the polymethine dye C.I. (Colour Index) Basic Yellow 11 (also known as Astrazon Yellow 5G) (Society of Dyers and Colourists, 1956b; Venkataraman, 1952). C.I. Basic Yellow 11 is widely used as a dye for polyacrylonitrile fibers and for printing on acetate and acetate/viscose rayon mixtures (Society of Dyers and Colourists, 1956a; Venkataraman, 1952).

*The CAS registry number is 54150-69-5.

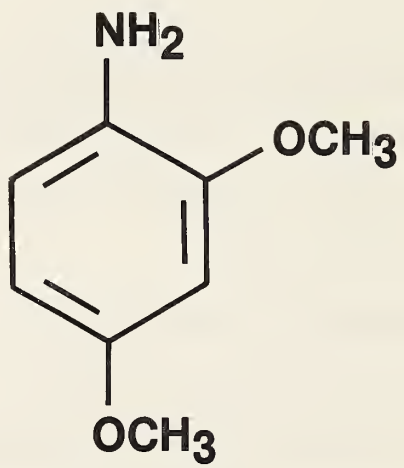


FIGURE 1
CHEMICAL STRUCTURE OF 2,4-DIMETHOXYANILINE (HYDROCHLORIDE)

Specific production data for 2,4-dimethoxyaniline hydrochloride and 2,4-dimethoxyaniline are not available; however, only the latter compound appears to be produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) in the United States (U.S. International Trade Commission, 1977). Imports of 2,4-dimethoxyaniline through principal U.S. customs districts amounted to 195,800 pounds in 1974 (U.S. International Trade Commission, 1976). In 1976, the last year for which data are available, production and sales of C.I. Basic Yellow 11 at five U.S. facilities were 885,000 and 737,000 pounds, respectively (U.S. International Trade Commission, 1977).

The potential for exposure to 2,4-dimethoxyaniline is greatest for workers in facilities which produce this compound or use it as an intermediate in the production of Basic Yellow 11. Some exposure of researchers to 2,4-dimethoxyaniline hydrochloride may also occur.

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade 2,4-dimethoxyaniline hydrochloride was purchased from Pharm-Eco Chemical Company. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. The experimentally determined melting point range of 33.5° to 37°C compares favorably to the value of 33.5°C reported in the literature (Weast, 1978). Thin-layer chromatography (TLC) was performed utilizing two solvent systems (i.e., diethyl ether:acetic acid:hexane and benzene:methanol). Each plate, visualized with ultraviolet and visible light, iodine vapor, and ferric chloride-potassium ferricyanide spray, revealed a single spot. Gas liquid chromatography (GLC) presented one homogeneous peak. The results of infrared (IR) and nuclear magnetic resonance (NMR) analyses were consistent with those reported in the literature (Sadtler Standard Spectra). Ultraviolet/visible (UV/VIS) analysis revealed λ_{max} at 235 and 295 nm with respective molar extinction coefficients (ϵ) of 8.6×10^3 and 3.5×10^3. Comparison with the literature values (Sadtler Standard Spectra) of λ_{max} at 235.5 and 296 nm with respective ϵ values of 8.2×10^3 and 3.8×10^3, indicated a compound of high purity.

A second batch of the compound was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Chemical analysis was performed by Midwest Research Institute. The manufacturer's stated purity was 97 percent. TLC was performed utilizing two solvent systems (i.e.,

ethanol:water and chloroform). Each plate was visualized with iodine vapor, furfural and UV light of 254 and 366 nm. Using the first solvent system, only one spot was revealed, while the plate developed with chloroform showed two spots, one major spot and a trace at the origin. Elemental analysis closely approximated that expected on the basis of the molecular formula of the compound. Titration of the amino group with perchloric acid was almost identical with the theoretical. Vapor phase chromatography showed a major peak with five minor peaks, accounting for less than 1 percent of the total area. The experimentally determined melting point range of 32° to 34°C closely approximated the value reported in the literature (Weast, 1978). The results of IR and NMR analyses were consistent with those reported in the literature (Sadtler Standard Spectra). UV/VIS analysis revealed λ_{max} at 236 and 297 nm with respective ϵ values of 7.5×10^3 and 3.3×10^3.

Throughout this report, the term 2,4-dimethoxyaniline HCl is used to represent this technical-grade material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). 2,4-Dimethoxyaniline HCl was administered to the dosed animals as a component of the diet.

The chemical was removed from its container and a weighed amount was blended with an aliquot of the ground feed using a mortar and

pestle. Once visual homogeneity was attained, the mixture was placed in a 6 kg capacity Patterson-Kelley standard model twin-shell stainless steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

Dosed feed preparations containing 1500 and 5000 ppm of 2,4-dimethoxyaniline HCl were analyzed spectrophotometrically for the compound. The mean result immediately after preparation was 100.4 percent of theoretical (ranging from 90.5 to 106.6 percent).

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. All rats and mice were supplied by the Frederick Cancer Research Center, Frederick, Maryland.

Rats and mice were approximately 4 weeks old when received. Upon receipt, animals were examined and obviously ill or runted animals were killed. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were placed on test at this time. Animals were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

All animals were housed by species in temperature- and humidity-controlled rooms. The temperature range was 22° to 26°C and the relative humidity was maintained between 45 and 55 percent. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia) at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

All rats were housed four per cage by sex and all mice were housed five per cage by sex. Throughout the study dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding were provided twice weekly. Ab-sorb-dri® hardwood chip bedding (Wilner Wood Products Company, Norway, Maine) was used in polycarbonate cages for the entire bioassay.

Acidulated water (pH 2.5) was supplied to animals in water bottles filled by an automated metering device that was checked daily for diluting accuracy. Water bottles were changed and washed twice weekly, and sipper tubes were washed at weekly intervals. During the period of chemical administration, dosed and control animals received treated or untreated Wayne Lab-Blox® meal as appropriate. The feed

was supplied in hanging stainless steel hoppers which were refilled three times per week and sanitized weekly. Food and water were available ad libitum for both species.

All dosed and control rats were housed in a room with other rats receiving diets containing* 4'-(chloroacetyl)-acetanilide (140-49-8) and nithiazide (139-94-6); and with other rats intubated with trimethylphosphate (572-56-1).

All dosed and control mice were housed in a room with mice receiving diets contaning 4'-(chloroacetyl)-acetanilide (140-49-8); nithiazide (139-94-6); p-phenylenediamine dihydrochloride (624-18-0); 4-nitro-o-phenylenediamine (99-56-9); 1-phenyl-3-methyl-5-pyrazolone (89-25-8); and other mice intubated with trimethylphosphate (512-56-1); 3-(chloromethyl)pyridine hydrocholoride (3099-31-8); 2-(chloromethyl)pyridine hydrocholoride (6959-17-3); and pivalolactone (1955-45-9).

E. Selection of Initial Concentrations

To establish the maximum tolerated concentrations of 2,4-dimethoxyaniline HCl for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Rats were distributed among nine groups, each consisting of five males and five females. 2,4-Dimethoxyaniline HCl was incorporated into the basal laboratory diet and supplied ad libitum to seven

*CAS registry numbers are given in parentheses.

of the nine rat groups in concentrations of 2150, 3160, 4640, 6800, 10,000, 14,700 and 21,600 ppm. The two remaining rat groups served as control groups, receiving only the basal laboratory diet.

Mice were distributed among nine groups, each consisting of five males and five females. 2,4-Dimethoxyaniline HCl was incorported into the basal laboratory diet and supplied ad libitum to seven of the nine mouse groups in concentrations of 3160, 4640, 6800, 10,000, 14,700, 21,600, and 31,500 ppm. The two remaining mouse groups served as control groups, receiving only the basal laboratory diet.

The dosed dietary preparations were administered for a period of 7 weeks, followed by a 1-week observation period during which all animals were fed the basal laboratory diet. Individual body weights and food consumption data were recorded twice weekly throughout the study. Upon termination of the study all survivors were sacrificed and necropsied.

The following table indicates the mean body weight gain, relative to controls, survival, and incidence of darkened thyroids observed in each of the rat groups at the end of the subchronic test.

RAT SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)*		Survival*		Observation of Darkened Thyroids**	
	Males	Females	Males	Females	Males	Females
21,600	-77	-34	5/5	5/5	5/5	5/5
14,700	-48	-17	5/5	5/5	5/5	5/5
10,000	-16	-15	5/5	5/5	5/5	5/5
6,800	- 5	-21	5/5	5/5	5/5	5/5
4,640	- 3	- 8	5/5	5/5	0/5	0/5
3,160	+15	-15	5/5	5/5	0/5	0/5
2,150	+25	-12	5/5	5/5	0/5	0/5
0	--	--	5/5	5/5	0/5	0/5

The high concentration selected for administration to dosed rats in the chronic bioassay was 3000 ppm.

The following table indicates the mean body weight gain, relative to controls, and survival observed in each of the mouse groups at the end of the subchronic test.

MOUSE SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)*		Survival**	
	Males	Females	Males	Females
31,500	-20	-31	5/5	2/5
21,600	-15	-32	5/5	2/5
14,700	-16	-25	5/5	5/5
10,000	- 5	-31	5/5	4/5
6,800	-14	-30	5/5	5/5
4,640	- 1	+10	5/5	5/5
3,160	- 5	+11	5/5	5/5
0	--	--	5/5	5/5

*+ is indicative of mean body weight gain greater than that of controls
- is indicative of mean body weight gain less than that of controls.
** Number of animals observed/number of animals originally in group.

No clinical signs were recorded for any mouse group. The high concentration selected for administration to dosed mice in the chronic bioassay was 5000 ppm.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered, and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dietary concentrations of 2,4-dimethoxyaniline HCl administered to rats were 3000 and 1500 ppm. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. Dosed rats were supplied with feed containing 2,4-dimethoxyaniline HCl for 104 weeks followed by a 1-week observation period.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dietary concentrations of 2,4-dimethoxyaniline HCl administered were 5000 and 2500 ppm. Throughout this report those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
2,4-DIMETHOXYANILINE HCl FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2,4-DIMETHOXY-ANILINE HCl CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	105
LOW DOSE	50	1500	104	
		0		1
HIGH DOSE	50	3000	104	
		0		1
FEMALE				
CONTROL	20	0	0	105
LOW DOSE	50	1500	104	
		0		1
HIGH DOSE	50	3000	104	
		0		1

[a]Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
2,4-DIMETHOXYANILINE HCl FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2,4-DIMETHOXY-ANILINE HCl CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	104
LOW DOSE	50	2500	103	
		0		1
HIGH DOSE	50	5000	103	
		0		1
FEMALE				
CONTROL	20	0	0	104
LOW DOSE	50	2500	103	
		0		1
HIGH DOSE	50	5000	103	
		0		1

[a]Concentrations given in parts per million.

groups. Dosed mice were supplied with feed containing 2,4-dimethoxyaniline HCl for 103 weeks followed by a 1-week observation period.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment and body weights were recorded at monthly intervals throughout the bioassay. All animals were inspected twice daily for mortality. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group.

All moribund animals or animals that developed large, palpable masses that jeopardized their health were sacrificed. A necropsy was performed on each animal regardless of whether it died, was sacrificed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized using carbon dioxide, and were immediately necropsied. Gross and microscopic examinations were performed on all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney,

urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were recorded in each group at the time that the test was initiated.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report

in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence

of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that

survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk

of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

No dose-related mean body weight depression was apparent in male rats until week 80. The mean body weight of the high dose males was slightly depressed, relative to the controls, starting in week 20 and continuing throughout the bioassay. Slight, although consistent, dose-related mean body weight depression was apparent in female rats throughout the bioassay (Figure 2).

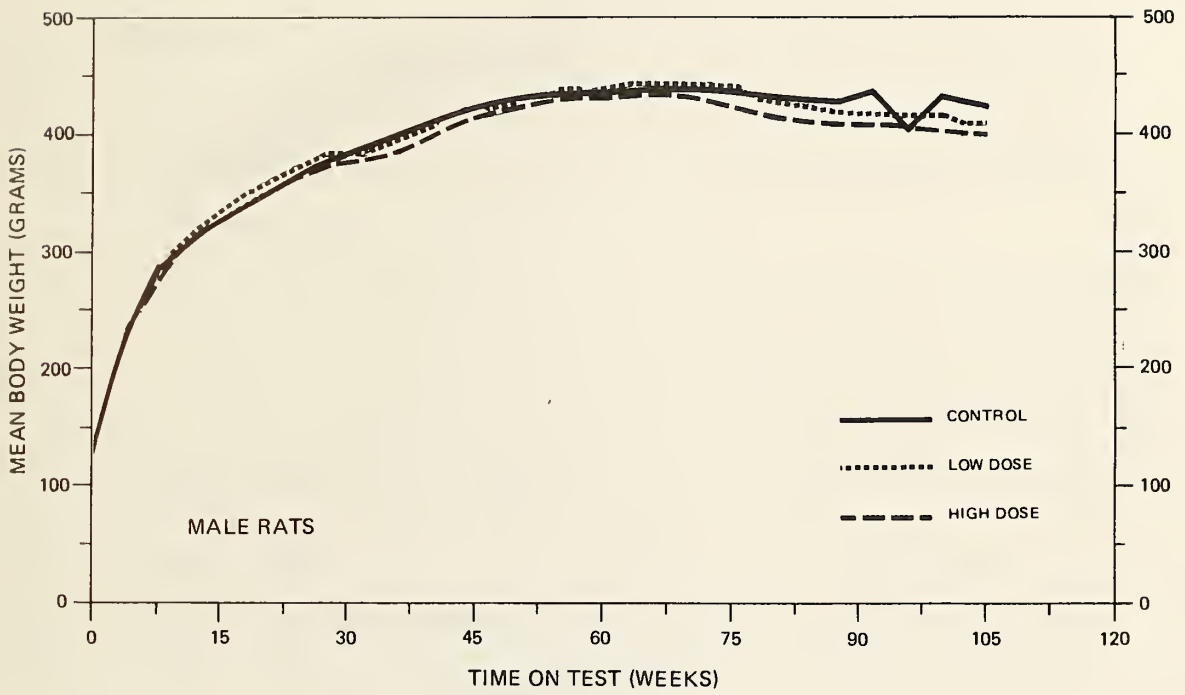
No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 2,4-dimethoxyaniline HCl-dosed groups are shown in Figure 3. For both males and females, the statistical tests indicated no significant positive associations between dosage and mortality. The Tarone test and the Cox tests indicated a significant negative association for female rats.

There were adequate numbers of male rats at risk from late-developing tumors as 90 percent (45/50) of the high dose, 94 percent (47/50) of the low dose, and 85 percent (17/20) of the controls survived on test for at least 90 weeks.

For females, with 96 percent (48/50) of the high dose, 92 percent (46/50) of the low dose, and 85 percent (17/20) of the controls surviving on test for at least 90 weeks, there were adequate numbers at risk from late-developing tumors.



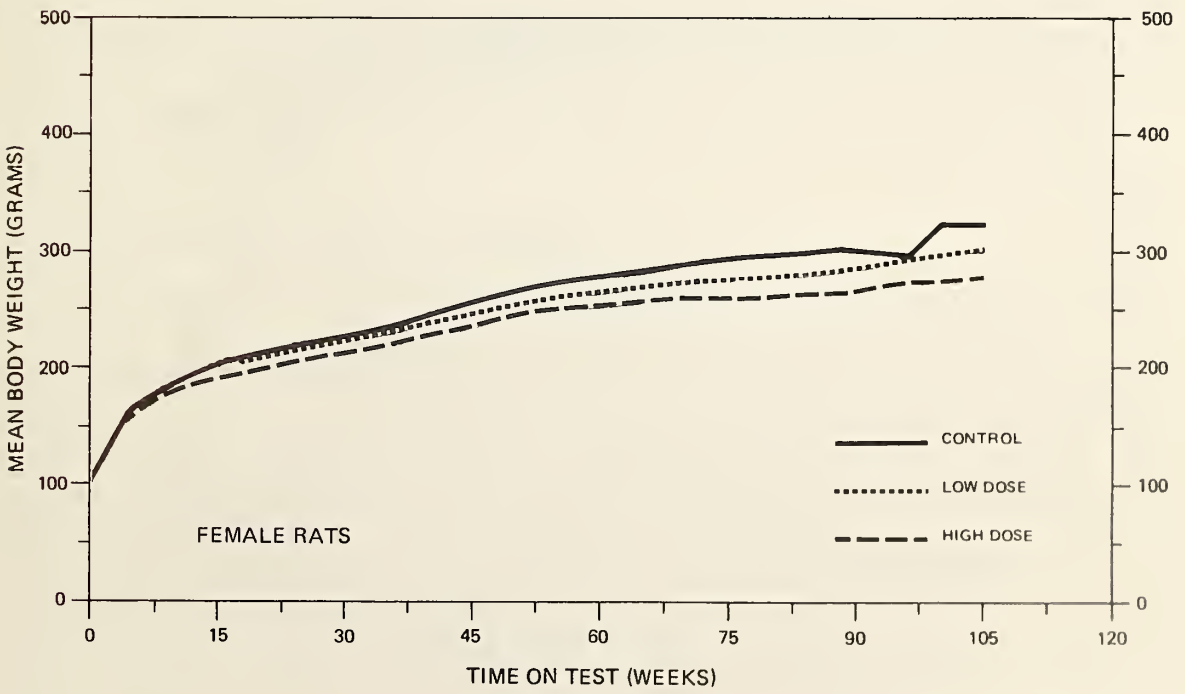
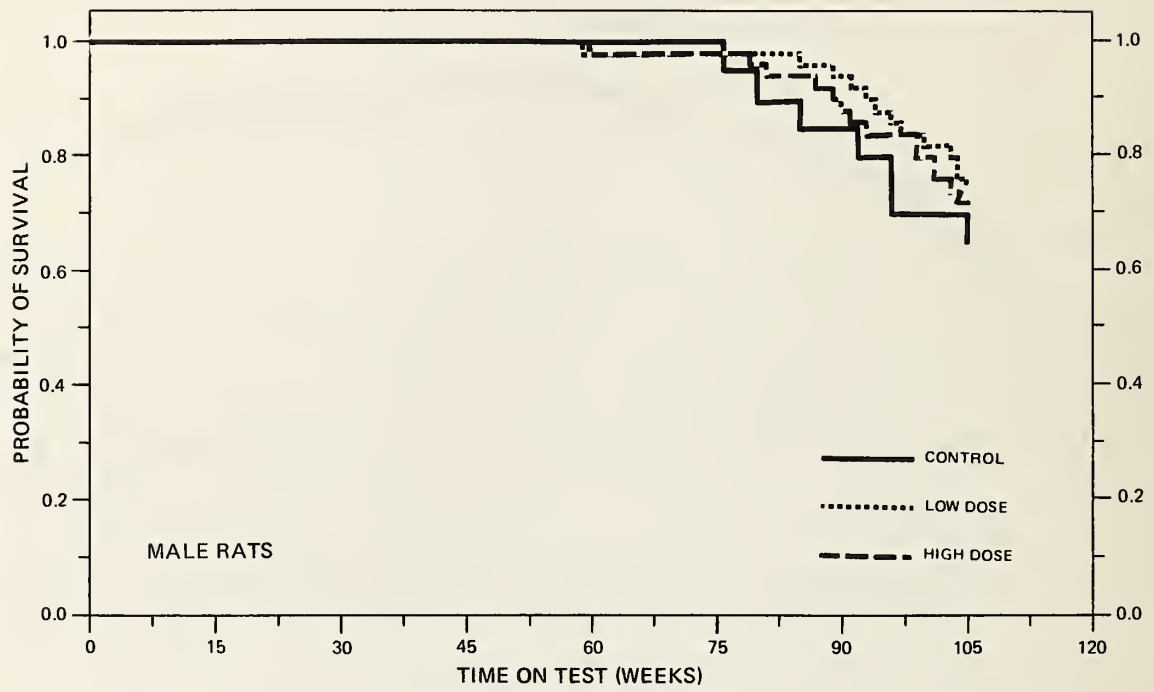


FIGURE 2
GROWTH CURVES FOR 2,4-DIMETHOXYANILINE HYDROCHLORIDE CHRONIC STUDY RATS



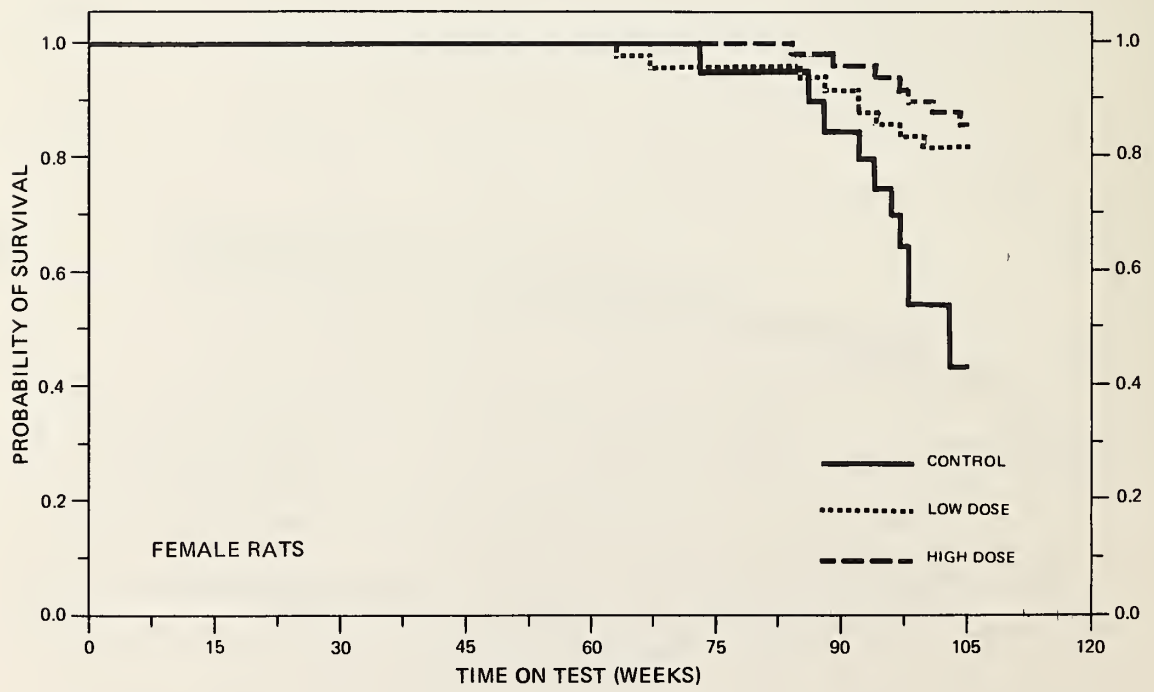


FIGURE 3
SURVIVAL COMPARISONS OF 2,4-DIMETHOXYANILINE HYDROCHLORIDE CHRONIC STUDY RATS

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2).

A variety of neoplasms was found in both dosed and control groups. Each of these neoplasms occurs spontaneously in aged Fischer 344 rats. Neither the general incidence of neoplasms nor any specific benign or malignant neoplasm occurred in either male or female rats in such numbers as to indicate direct compound effect.

A variety of nonneoplastic lesions occurred in both dosed and control rats in about equal proportions and were judged to be spontaneous.

Based on the results of this pathologic examination, 2,4-dimethoxyaniline HCl was not carcinogenic in Fischer 344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 2,4-dimethoxyaniline HCl-dosed groups and where such tumors were observed in at least 5 percent of the group.

None of the statistical tests for any site in the rats of either sex indicated a significant positive association between chemical

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	2/20(0.10)	2/50(0.04)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.400	0.200
Lower Limit	---	0.032	0.004
Upper Limit	---	5.277	3.681
Weeks to First Observed Tumor	105	105	105
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/20(0.15)	16/50(0.32)	7/50(0.14)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.028	---	---
Relative Risk (Control)[d]	---	2.133	0.933
Lower Limit	---	0.716	0.245
Upper Limit	---	10.524	5.215
Weeks to First Observed Tumor	85	59	60
Pituitary: Chromophobe Carcinoma or Chromophobe Adenoma[b]	1/17(0.06)	3/43(0.07)	8/40(0.20)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.186	3.400
Lower Limit	---	0.106	0.524
Upper Limit	---	60.801	146.349
Weeks to First Observed Tumor	105	104	91

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma[b]	2/20(0.10)	4/50(0.08)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.800	0.600
Lower Limit	---	0.128	0.076
Upper Limit	---	8.436	6.860
Weeks to First Observed Tumor	105	97	101
Thyroid: C-Cell Adenoma[b]	0/20(0.00)	2/49(0.04)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.125	0.261
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	101
Pancreatic Islets: Islet-Cell Carcinoma or Islet-Cell Adenoma[b]	3/20(0.15)	1/50(0.02)	1/48(0.02)
P Values[c]	P = 0.045(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.133	0.139
Lower Limit	---	0.003	0.003
Upper Limit	---	1.568	1.631
Weeks to First Observed Tumor	80	104	105

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Testis: Interstitial-Cell Tumor or Interstitial-Cell Tumor, Malignant[b]	18/20(0.90)	46/49(0.94)	40/50(0.80)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.043	0.889
Lower Limit	---	0.916	0.775
Upper Limit	---	1.245	1.190
Weeks to First Observed Tumor	80	85	79
Body Cavities: Mesothelioma NOS[b]	1/20(0.05)	1/50(0.02)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.400	1.200
Lower Limit	---	0.005	0.106
Upper Limit	---	30.802	61.724
Weeks to First Observed Tumor	105	105	89

[a]Treated groups received doses of 1500 or 3000 ppm in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	6/19(0.32)	7/50(0.14)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.443	0.380
Lower Limit	---	0.153	0.121
Upper Limit	---	1.427	1.274
Weeks to First Observed Tumor	88	85	84
Pituitary: Chromophobe Carcinoma or Chromophobe Adenoma[b]	5/17(0.29)	14/49(0.29)	9/46(0.20)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.971	0.665
Lower Limit	---	0.409	0.245
Upper Limit	---	3.048	2.248
Weeks to First Observed Tumor	86	67	84
Mammary Gland: Fibroadenoma[b]	3/19(0.16)	3/50(0.06)	1/50(0.02)
P Values[c]	P = 0.036(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.380	0.127
Lower Limit	---	0.057	0.003
Upper Limit	---	2.658	1.487
Weeks to First Observed Tumor	73	105	97

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	4/19(0.21)	4/49(0.08)	4/49(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.388	0.388
Lower Limit	---	0.083	0.083
Upper Limit	---	1.917	1.917
Weeks to First Observed Tumor	98	92	104

[a]Treated groups received doses of 1500 or 3000 ppm in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

administration and tumor incidence. Based upon these statistical results there was no evidence that 2,4-dimethoxyaniline HCl was a carcinogen in Fischer 344 rats under the conditions of this bioassay.

In male rats the Cochran-Armitage test indicated a significant negative association between dose and the combined incidence of islet-cell carcinomas or islet-cell adenomas of the pancreas. The Cochran-Armitage test also indicated a significant negative association between dose and the incidence of fibroadenomas of the mammary gland in female rats.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by 2,4-dimethoxyaniline HCl that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

No dose-related mean body weight depression was apparent in male mice, although the mean body weight of the dosed groups was less than that of the controls throughout a major portion of the bioassay. Female mice evidenced distinct and consistent dose-related mean body weight depression throughout the bioassay (Figure 4).

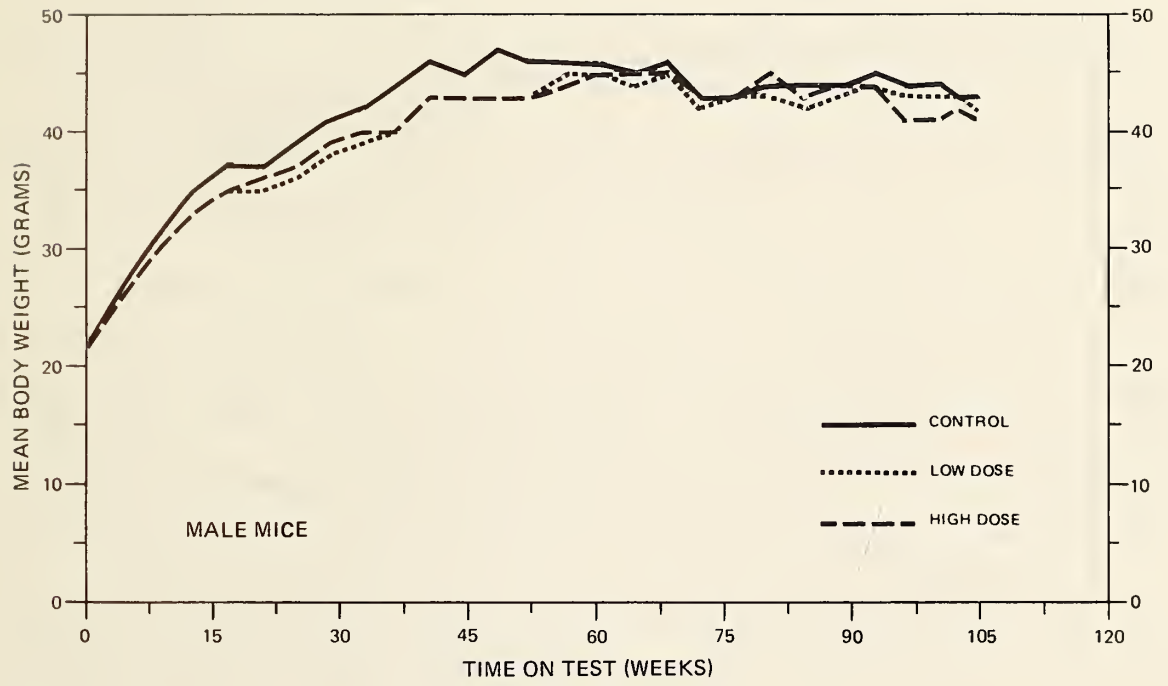
No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 2,4-dimethoxyaniline HCl-dosed groups are shown in Figure 5. Neither the Tarone test nor the Cox tests indicated a significant positive association between dosage and mortality in either male or female mice.

There were adequate numbers of male mice at risk from late-developing tumors, as 92 percent (46/50) of the high dose, 94 percent (47/50) of the low dose and 85 percent (17/20) of the controls survived on test for at least 90 weeks.

For females, with 88 percent (44/50) of the high dose, 90 percent (45/50) of the low dose and 95 percent (19/20) of the controls surviving on test for at least 90 weeks, there were adequate numbers at risk from late-developing tumors.



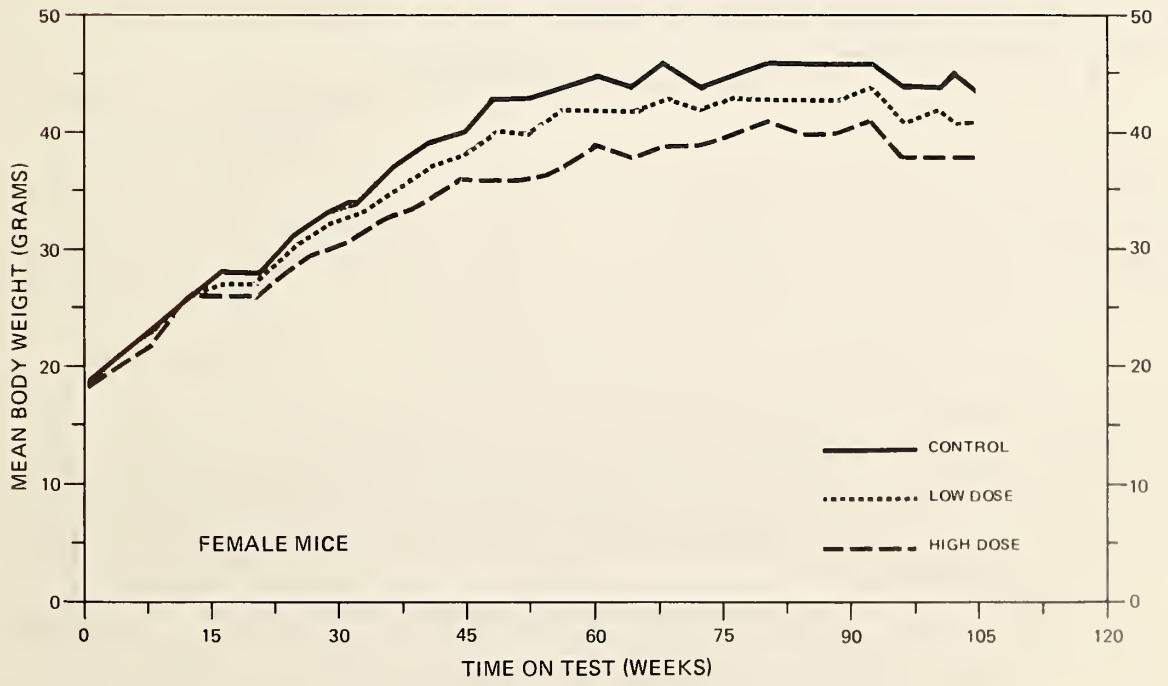


FIGURE 4
GROWTH CURVES FOR 2,4-DIMETHOXYANILINE HYDROCHLORIDE CHRONIC STUDY MICE

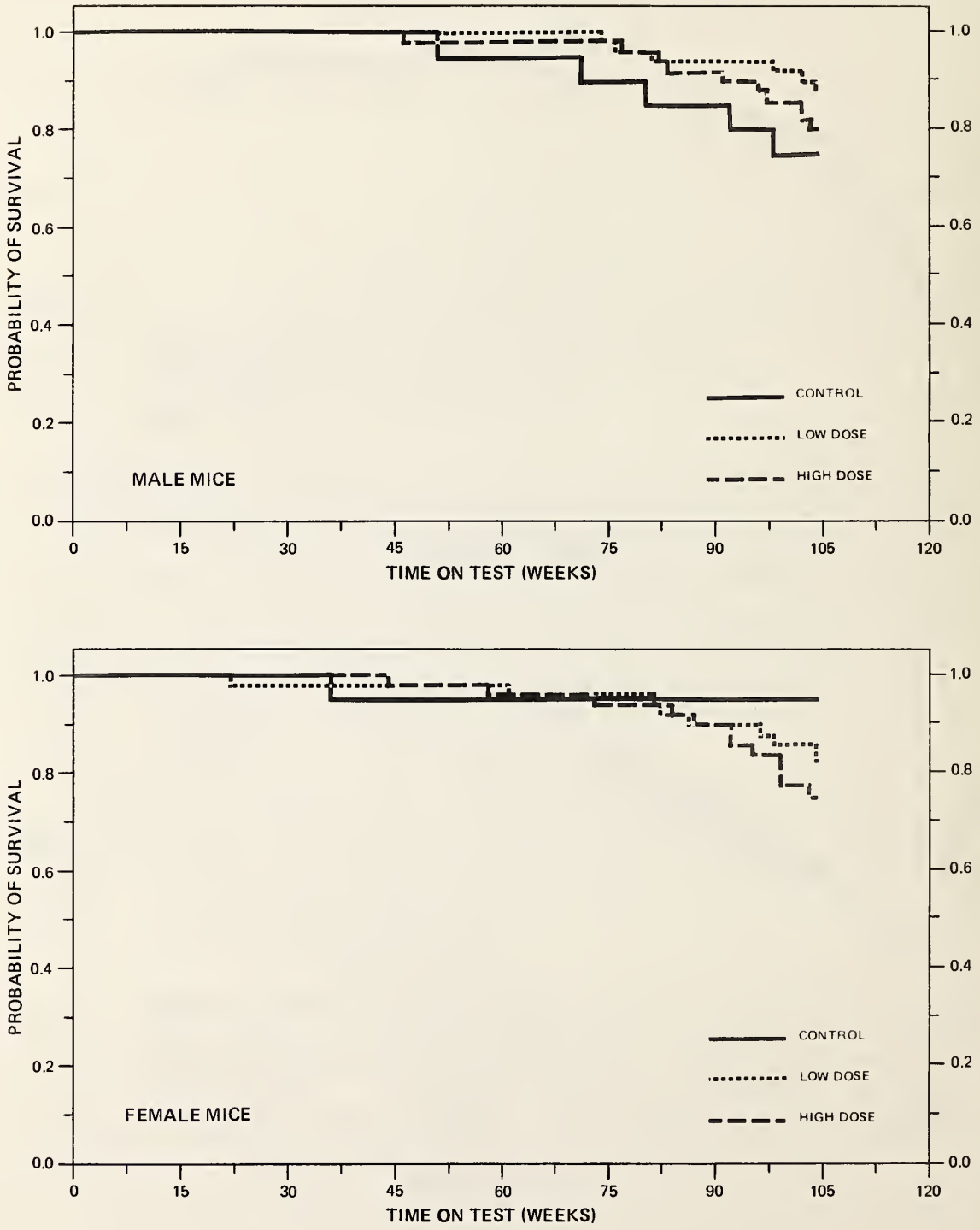


FIGURE 5
SURVIVAL COMPARISONS OF 2,4-DIMETHOXYANILINE HYDROCHLORIDE CHRONIC STUDY MICE

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2).

A variety of neoplasms and nonneoplastic lesions occurred in both dosed and control mice. All of these lesions have been observed in aged B6C3F1 mice. They did not appear to be related to compound administration except for proliferative thyroid and hepatic lesions.

There was an increased incidence of proliferative thyroid lesions in male and female mice when compared to their respective controls. The incidences are summarized below:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Animals with Thyroids Examined Histopathologically	(8)	(23)	(39)	(10)	(35)	(36)
Follicular-Cell Adenoma	0	0	4(10%)	0	1(3%)	2(6%)
Follicular-Cell Hyperplasia	0	0	3(8%)	0	0	2(6%)
Cystic Follicles	0	1(4%)	1(3%)	0	3(9%)	1(3%)

There was also an increased incidence of hepatocellular adenomas and hepatocellular carcinomas in the high dose males when compared to controls. Hepatocellular adenomas were observed in 11/50 (22 percent) and 2/20 (10 percent) of the high dose and control males, respectively, while hepatocellular carcinomas were observed in 16/50 (32 percent) and 5/20 (25 percent) of the high dose and control males, respectively.

Based on the results of this pathologic examination, administration of 2,4-dimethoxyaniline HCl was associated with an increased incidence of proliferative thyroid lesions in B6C3F1 mice of both sexes and with liver neoplasms in high dose male mice under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 2,4-dimethoxyaniline HCl-dosed groups and where such tumors were observed in at least 5 percent of the group.

In male mice the Cochran-Armitage test indicated a significant ($P = 0.012$) positive association between dosage and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas. However, neither of the Fisher exact tests was significant and the test for departure from linear trend was significant ($P = 0.006$). Historical control data from the same laboratory indicate a combined incidence of 16 percent (54/340) for hepatocellular carcinomas and hepatocellular adenomas in untreated control B6C3F1 male mice as compared to the 35 percent (7/20) observed in control males in this bioassay.

None of the statistical tests at any site, including the thyroid, indicated a significant positive association between dosage and tumor incidence for female mice.

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	4/20(0.20)	6/48(0.13)	2/49(0.04)
P Values[c]	P = 0.031(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.625	0.204
Lower Limit	---	0.171	0.020
Upper Limit	---	2.764	1.323
Weeks to First Observed Tumor	71	98	82
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	3/50(0.06)	5/50(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.600	1.000
Lower Limit	---	0.076	0.184
Upper Limit	---	6.860	10.007
Weeks to First Observed Tumor	51	74	96
Liver: Hepatocellular Carcinoma[b]	5/20(0.25)	4/49(0.08)	16/50(0.32)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.011	---	---
Relative Risk (Control)[d]	---	0.327	1.280
Lower Limit	---	0.074	0.538
Upper Limit	---	1.385	3.983
Weeks to First Observed Tumor	71	98	46

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	7/20(0.35)	9/49(0.18)	27/50(0.54)
P Values[c]	P = 0.012	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.006	---	---
Relative Risk (Control)[d]	---	0.525	1.543
Lower Limit	---	0.211	0.818
Upper Limit	---	1.464	3.545
Weeks to First Observed Tumor	71	98	46
Thyroid: Follicular-Cell Adenoma[b]	0/08(0.00)	0/23(0.00)	4/39(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.219
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	104

[a]Treated groups received doses of 2500 or 5000 ppm in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	10/49(0.20)	7/48(0.15)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.041	1.458
Lower Limit	---	0.498	0.316
Upper Limit	---	18.154	13.664
Weeks to First Observed Tumor	104	61	87
Liver: Hepatocellular Carcinoma[b]	0/20(0.00)	4/49(0.08)	0/47(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.022	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.394	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	104	---
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	3/20(0.15)	12/49(0.24)	11/47(0.23)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.633	1.560
Lower Limit	---	0.513	0.480
Upper Limit	---	8.342	8.051
Weeks to First Observed Tumor	104	104	99

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Adenoma[b]	0/10(0.00)	1/35(0.03)	2/36(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.017	0.091
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	104

[a]Treated groups received doses of 2500 or 5000 ppm in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

For male mice the Cochran-Armitage test indicated a significant negative association between dosage and the combined incidence of alveolar/bronchiolar carcinomas or alveolar/bronchiolar adenomas. However, neither of the Fisher exact tests was significant.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by 2,4-dimethoxyaniline HCl that could not be established under the conditions of this test.

V. DISCUSSION

There were no significant positive associations between the concentrations of 2,4-dimethoxyaniline HCl administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Dose-related mean body weight depression was observed for females of both species, indicating that the concentrations of 2,4-dimethoxyaniline HCl administered to these groups may have approximated the maximum tolerated concentrations. Compound-related mean body weight depression was only slight for male rats and was apparent in male mice only until week 50; however, follicular-cell hyperplasias and cystic follicles of the thyroid were observed in dosed male mice, an indication that the concentrations the male mice received may have approximated the maximum tolerated concentrations. Since no distinct mean body weight depression in relation to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of 2,4-dimethoxyaniline HCl to male rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

None of the statistical tests for any site in rats of either sex or in female mice indicated a significant positive association between compound administration and tumor incidence. There was a significant positive association between concentration and the incidence of a combination of hepatocellular carcinomas and hepatocellular

adenomas in male mice; however, the Fisher exact comparisons were not significant. Although follicular-cell adenomas were observed in mice of both sexes, and only in dosed mice, the incidences of these neoplasms were not significantly higher in the dosed groups than in the controls.

Under the conditions of this bioassay there was no convincing evidence for the carcinogenicity of 2,4-dimethoxyaniline HCl in Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH
2,4-DIMETHOXYANILINE HYDROCHLORIDE

	CONTROL(UNTR) 11-1175	LOW DOSE 11-1173	HIGH DOSE 11-1171
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
TRICHOEPITHELIOMA	1 (5%)	2 (4%)	2 (4%)
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROSARCOMA			1 (2%)
NEUROFIBROMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	2 (4%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
ADENOCA/SQUAMOUS METAPLASIA	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMIA,NOS		2 (4%)	
UNDIFFERENTIATED LEUKEMIA	3 (15%)	10 (20%)	7 (14%)
#BONE MARROW	(20)	(49)	(49)
UNDIFFERENTIATED LEUKEMIA		1 (2%)	
#SPLEEN	(20)	(49)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
UNDIFFERENTIATED LEUKEMIA		2 (4%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1175	LOW DOSE 11-1173	HIGH DOSE 11-1171
DIGESTIVE SYSTEM			
#JEJUNUM	(20)	(47)	(49)
LEIOMYOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
INTERSTITIAL-CELL TUMOR, METASTA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(43)	(40)
CHROMOPHOBE ADENOMA	1 (6%)	3 (7%)	7 (18%)
CHROMOPHOBE CARCINOMA			1 (3%)
#ADRENAL	(20)	(50)	(50)
CORTICAL ADENOMA		1 (2%)	
PHEOCHROMOCYTOMA	2 (10%)	4 (8%)	3 (6%)
#THYROID	(20)	(49)	(48)
FOLLICULAR-CELL ADENOMA			2 (4%)
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA		2 (4%)	3 (6%)
CYSTADENOMA, NOS		1 (2%)	1 (2%)
#PARATHYROID	(11)	(35)	(33)
ADENOMA, NOS		1 (3%)	
#PANCREATIC ISLETS	(20)	(50)	(48)
ISLET-CELL ADENOMA	2 (10%)	1 (2%)	1 (2%)
ISLET-CELL CARCINOMA	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA		2 (4%)	
#TESTIS	(20)	(49)	(50)
INTERSTITIAL-CELL TUMOR	18 (90%)	46 (94%)	39 (78%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1175	LOW DOSE 11-1173	HIGH DOSE 11-1171
INTERSTITIAL-CELL TUMOR, MALIGNA			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(19)	(50)	(49)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*TARSAL GLAND	(20)	(50)	(50)
ADENOCARCINOMA, NOS	1 (5%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
*PERITONEUM	(20)	(50)	(50)
MESOTHELIOMA, NOS	1 (5%)		1 (2%)
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	8	10
MORIBUND SACRIFICE	2	5	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	37	36
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 11-1175	LOW DOSE 11-1173	HIGH DOSE 11-1171
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	49	48
TOTAL PRIMARY TUMORS	34	85	73
TOTAL ANIMALS WITH BENIGN TUMORS	18	48	44
TOTAL BENIGN TUMORS	27	65	59
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	18	10
TOTAL MALIGNANT TUMORS	6	19	11
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	1	3
TOTAL UNCERTAIN TUMORS	1	1	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH
2,4-DIMETHOXYANILINE HYDROCHLORIDE

	CONTROL(UNTR) 11-1176	LOW DOSE 11-1174	HIGH DOSE 11-1172
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	19	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(19)	(50)	(50)
TRICHOEPITHELIOMA		2 (4%)	
SEBACEOUS ADENOCARCINOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(18)	(48)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
UNDIFFERENTIATED LEUKEMIA	3 (16%)	5 (10%)	4 (8%)
LYMPHOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(19)	(48)	(49)
UNDIFFERENTIATED LEUKEMIA	2 (11%)		1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1176	LOW DOSE 11-1174	HIGH DOSE 11-1172
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(49)	(46)
CHROMOPHOBE ADENOMA	4 (24%)	13 (27%)	9 (20%)
CHROMOPHOBE CARCINOMA	1 (6%)	1 (2%)	
#ADRENAL	(19)	(49)	(50)
CORTICAL ADENOMA		2 (4%)	
PHECCHROMOCYTOMA	1 (5%)		
PHECCHROMOCYTOMA, MALIGNANT		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(19)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	2 (4%)
FIBROADENOMA	3 (16%)	3 (6%)	1 (2%)
#UTERUS	(19)	(49)	(49)
LEIOMYOSARCOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	4 (21%)	4 (8%)	4 (8%)
NERVOUS SYSTEM			
#BRAIN	(19)	(49)	(50)
CHROMOPHOBE CARCINOMA, INVASIVE	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(19)	(50)	(50)
MESOTHELIOMA, NOS	1 (5%)		
*MESENTERY	(19)	(50)	(50)
LIPOMA		1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONCLUDED)

	CONTROL(UNTR) 11-1176	LOW DOSE 11-1174	HIGH DOSE 11-1172
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
SARCOMA, NOS		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	3	3
MORIBUND SACRIFICE	6	6	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	8	41	43
ANIMAL MISSING	1		
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	16	31	21
TOTAL PRIMARY TUMORS	20	39	25
TOTAL ANIMALS WITH BENIGN TUMORS	12	25	14
TOTAL BENIGN TUMORS	12	27	16
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	12	9
TOTAL MALIGNANT TUMORS	7	12	9
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1		
TOTAL UNCERTAIN TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE

	CONTROL (UNTR) 22-2175	LOW DOSE 22-2173	HIGH DOSE 22-2171
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(49)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (15%)	6 (13%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)		
SARCOMA, NOS	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (10%)	1 (2%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE			2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	1 (2%)
#SPLEEN	(19)	(48)	(49)
HEMANGIOSARCOMA		1 (2%)	
#MESENTERIC L. NODE	(18)	(45)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
CIRCULATORY SYSTEM			
*PULMONARY ARTERY	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL(UNIR) 22-2175	LOW DOSE 22-2173	HIGH LOSE 22-2171
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
HEPATOCELLULAR ADENOMA	2 (10%)	5 (10%)	11 (22%)
HEPATOCELLULAR CARCINOMA	5 (25%)	4 (8%)	16 (32%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(17)	(49)	(47)
PHEOCHROMOCYTOMA	1 (6%)		
#THYROID	(8)	(23)	(39)
FOLLICULAR-CELL ADENOMA			4 (10%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONCLUDED)

	CONTROL(UNTR) 22-2175	LOW DOSE 22-2173	HIGH DOSE 22-2171
ALL OTHER SYSTEMS			
SITE UNKNOWN			
ADENOCARCINOMA, NOS, METASTATIC			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	5	7
MORIBUND SACRIFICE		1	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	44	40
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	15	33
TOTAL PRIMARY TUMORS	15	19	38
TOTAL ANIMALS WITH BENIGN TUMORS	6	10	15
TOTAL BENIGN TUMORS	6	11	17
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	7	20
TOTAL MALIGNANT TUMORS	9	8	21
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	2
TOTAL SECONDARY TUMORS	1	1	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH
2,4-DIMETHOXYANILINE HYDROCHLORIDE

	CONTROL(UNTR) 22-2176	LOW DOSE 22-2174	HIGH DOSE 22-2172
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	20	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(48)
SARCOMA, NOS		2 (4%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(47)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)		2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
SARCOMA, NOS, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(48)
MALIGNANT LYMPHOMA, NOS		5 (10%)	3 (6%)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (4%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
MALIGNANT LYMPHOMA, MIXED TYPE		2 (4%)	1 (2%)
PLASMA-CELL TUMOR			1 (2%)
PLASMACYTIC LEUKEMIA			1 (2%)
#LIVER	(20)	(49)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(47)
HEPATOCELLULAR ADENOMA	3 (15%)	8 (16%)	11 (23%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2176	LOW DOSE 22-2174	HIGH DOSE 22-2172
HEPATOCELLULAR CARCINOMA		4 (8%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(10)	(35)	(36)
FOLLICULAR-CELL ADENOMA		1 (3%)	2 (6%)
REPRODUCTIVE SYSTEM			
#OVARY	(17)	(32)	(27)
CYSTADENOMA, NOS			1 (4%)
PAPILLARY CYSTADENOMA, NOS		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL WALL	(20)	(49)	(48)
SARCOMA, NOS			1 (2%)
*MESENTERY	(20)	(49)	(48)
SARCOMA, NOS, METASTATIC		1 (2%)	
ALL OTHER SYSTEMS			
SITE UNKNOWN			
SARCOMA, NOS			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONCLUDED)

	CONTROL(UNTR) 22-2176	LOW DOSE 22-2174	HIGH DOSE 22-2172
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	7	12
MORIBUND SACRIFICE		2	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	40	36
ANIMAL MISSING		1	2
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	25	24
TOTAL PRIMARY TUMORS	6	27	26
TOTAL ANIMALS WITH BENIGN TUMORS	4	10	15
TOTAL BENIGN TUMORS	4	10	16
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	15	9
TOTAL MALIGNANT TUMORS	2	17	9
TOTAL ANIMALS WITH SECONDARY TUMORS#		3	
TOTAL SECONDARY TUMORS		3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH
2,4-DIMETHOXYANILINE HYDROCHLORIDE

	CONTROL(UNTR) 11-1175	LOW DOSE 11-1173	HIGH DOSE 11-1171
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
NECROSIS, FAT	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
#LUNG	(20)	(50)	(50)
PNEUMONIA, CHRONIC MURINE	2 (10%)	2 (4%)	3 (6%)
FIBROSIS, FOCAL		1 (2%)	
HYPERPLASIA, ADENOMATOUS			3 (6%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(49)	(49)
APLASIA, HEMATOPOIETIC		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(50)
THROMBUS, ORGANIZED	2 (10%)		
#MYOCARDIUM	(20)	(50)	(50)
FIBROSIS	5 (25%)	5 (10%)	5 (10%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
CIRRHOSIS, BILIARY	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 11-1175	LOW DOSE 11-1173	HIGH DOSE 11-1171
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL	2 (10%)		1 (2%)
NECROSIS, CENTRAL			2 (4%)
METAMORPHOSIS FATTY		2 (4%)	1 (2%)
#LIVER/CENTRILOBULAR	(20)	(49)	(50)
NECROSIS, NOS		1 (2%)	
METAMORPHOSIS FATTY			2 (4%)
#BILE DUCT	(20)	(49)	(50)
HYPERPLASIA, NOS	4 (20%)	4 (8%)	2 (4%)
#PANCREAS	(20)	(50)	(48)
FIBROSIS, FOCAL	1 (5%)	2 (4%)	1 (2%)
#CARDIAC STOMACH	(20)	(49)	(48)
ULCER, NOS		1 (2%)	
#COLON	(20)	(49)	(49)
NEMATODIASIS	3 (15%)	12 (24%)	17 (35%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
INFLAMMATION, CHRONIC	11 (55%)	29 (58%)	21 (42%)
#URINARY BLADDER	(19)	(49)	(46)
INFLAMMATION, HEMORRHAGIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(43)	(40)
HYPERPLASIA, CHROMOPHOBE-CELL		1 (2%)	
#ADRENAL	(20)	(50)	(50)
NECROSIS, FOCAL		1 (2%)	
#ADRENAL MEDULLA	(20)	(50)	(50)
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(20)	(49)	(46)
HYPERPLASIA, C-CELL		1 (2%)	2 (4%)
#PARATHYROID	(11)	(35)	(33)
HYPERPLASIA, NOS			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL(UNTR) 11-1175	LOW DOSE 11-1173	HIGH DOSE 11-1171
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
NECROSIS, FOCAL			1 (2%)
HYPERPLASIA, NOS			1 (2%)
*SEMINAL VESICLE	(20)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
#TESTIS	(20)	(49)	(50)
ATROPHY, NOS	4 (20%)	1 (2%)	9 (18%)
NERVOUS SYSTEM			
#BRAIN	(19)	(50)	(49)
COMPRESSION			2 (4%)
INFARCT, NOS	1 (5%)	1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
PERIARTERITIS			1 (2%)
*MESENTERY	(20)	(50)	(50)
STEATITIS			1 (2%)
NECROSIS, FAT		2 (4%)	3 (6%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE

	CONTROL (UNTR) 11-1176	LOW DOSE 11-1174	HIGH DOSE 11-1172
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	19	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(19)	(50)	(50)
ULCER, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(18)	(48)	(50)
EDEMA, NOS		1 (2%)	
PNEUMONIA, ASPIRATION			1 (2%)
PNEUMONIA, CHRONIC MURINE	2 (11%)	1 (2%)	5 (10%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
#LUNG/ALVEOLI	(18)	(48)	(50)
HYPERTROPHY, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(46)	(45)
APLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(19)	(48)	(49)
HYPERPLASIA, NOS		1 (2%)	
#LYMPH NODE	(19)	(48)	(46)
FIBROSIS		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(49)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
FIBROSIS		3 (6%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL(UNIF) 11-1176	LOW DOSE 11-1174	HIGH DOSE 11-1172
DIGESTIVE SYSTEM			
#LIVER	(19)	(48)	(50)
CIRRHOSIS, BILIARY	1 (5%)		
NECROSIS, FOCAL	1 (5%)		
METAMORPHOSIS FATTY	4 (21%)	1 (2%)	
BASOPHILIC CYTO CHANGE			1 (2%)
#LIVER/PERIPORTAL	(19)	(48)	(50)
FIBROSIS, FOCAL		1 (2%)	
#BILE DUCT	(19)	(48)	(50)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
#PANCREAS	(18)	(49)	(48)
FIBROSIS		1 (2%)	
FIBROSIS, FOCAL		1 (2%)	
#PANCREATIC DUCT	(18)	(49)	(48)
HYPERPLASIA, NOS		1 (2%)	
#STOMACH	(19)	(47)	(49)
ULCER, NOS		1 (2%)	
#COLON	(17)	(48)	(48)
NEMATODIASIS	5 (29%)	13 (27%)	14 (29%)
URINARY SYSTEM			
#KIDNEY	(19)	(49)	(50)
INFLAMMATION, CHRONIC		4 (8%)	2 (4%)
#URINARY BLADDER	(18)	(47)	(48)
CALCULUS, NOS	1 (6%)		
INFLAMMATION PROLIFERATIVE	1 (6%)		
METAPLASIA, SQUAMOUS	1 (6%)		
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(49)	(46)
CYST, NOS		2 (4%)	1 (2%)
HYPERPLASIA, CHROMOPHOBE-CELL	1 (6%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1176	LOW DOSE 11-1174	HIGH DOSE 11-1172
#ADRENAL	(19)	(49)	(50)
METAMORPHOSIS FATTY			2 (4%)
#ADRENAL CORTEX	(19)	(49)	(50)
NECROSIS, NOS			1 (2%)
#THYROID	(19)	(47)	(49)
HYPERPLASIA, C-CELL		5 (11%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(19)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	
#UTERUS	(19)	(49)	(49)
HYDROMETRA	2 (11%)	4 (8%)	9 (18%)
#UTERUS/ENDOMETRIUM	(19)	(49)	(49)
INFLAMMATION, SUPPURATIVE	1 (5%)		
#OVARY	(18)	(49)	(49)
CYST, NOS	1 (6%)	2 (4%)	2 (4%)
NERVOUS SYSTEM			
#CEREBRUM	(19)	(49)	(50)
COMPRESSION		1 (2%)	
#BRAIN	(19)	(49)	(50)
COMPRESSION		3 (6%)	4 (8%)
HYDROCEPHALUS, NOS		1 (2%)	
HEMORRHAGE	1 (5%)		1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONCLUDED)

	CONTROL(UNTR) 11-1176	LOW DOSE 11-1174	HIGH DOSE 11-1172
BODY CAVITIES			
*MESENTERY	(19)	(50)	(50)
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		9	8
ANIMAL MISSING/NO NECROPSY	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH
2,4-DIMETHOXYANILINE HYDROCHLORIDE

	CONTROL (UNTR) 22-2175	LOW DOSE 22-2173	HIGH DOSE 22-2171
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(49)
CONGESTION, NOS		3 (6%)	2 (4%)
EDEMA, NOS		1 (2%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	6 (30%)	13 (27%)	18 (37%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(48)	(49)
HYPERPLASIA, LYMPHOID	1 (5%)	3 (6%)	
#MESENTERIC L. NODE	(18)	(45)	(49)
CONGESTION, NOS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(49)	(47)
PERIARTERITIS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 22-2175	LOW DOSE 22-2173	HIGH DOSE 22-2171
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
INFARCT, NOS			1 (2%)
METAMORPHOSIS FATTY			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	2 (4%)
HYPERPLASIA, DIFFUSE			2 (4%)
ANGIECTASIS	1 (5%)		
#LIVER/PERIPORTAL	(20)	(49)	(50)
INFLAMMATION, CHRONIC			1 (2%)
#ESOPHAGUS	(12)	(45)	(47)
IMPACTION, NOS		1 (2%)	
INFLAMMATION, ACUTE NECROTIZING		1 (2%)	
#STOMACH	(17)	(46)	(50)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#LARGE INTESTINE	(18)	(48)	(50)
PARASITISM	5 (28%)	12 (25%)	24 (48%)
URINARY SYSTEM			
#KIDNEY	(19)	(49)	(49)
HYDRONEPHROSIS			1 (2%)
INFLAMMATION, CHRONIC			2 (4%)
GLOMERULONEPHRITIS, CHRONIC	1 (5%)		
INFLAMMATION, CHRONIC FOCAL		1 (2%)	1 (2%)
NEPHROSIS, NOS		1 (2%)	
AMYLOIDOSIS		1 (2%)	
METAPLASIA, OSSEOUS		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(17)	(49)	(47)
DEGENERATION, NOS			1 (2%)
#THYROID	(8)	(23)	(39)
CYSTIC FOLLICLES		1 (4%)	1 (3%)
INFLAMMATION, FOCAL			1 (3%)
HYPERPLASIA, FOLLICULAR-CELL			3 (8%)
#PARATHYROID	(3)	(1)	(7)
FIBROSIS, DIFFUSE			1 (14%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2175	LOW DOSE 22-2173	HIGH DOSE 22-2171
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
CONGESTION, NOS			1 (2%)
CORPORA AMYLACEA	8 (40%)	4 (8%)	19 (38%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT	2 (10%)	3 (6%)	1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	15	1
AUTO/NECROPSY/HISTO PERF		1	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH 2,4-DIMETHOXYANILINE HYDROCHLORIDE

	CONTROL(UNTR) 22-2176	LOW DOSE 22-2174	HIGH DOSE 22-2172
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	20	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(47)
CONGESTION, NOS		2 (4%)	
BRONCHOPNEUMONIA, NOS			2 (4%)
PNEUMONIA, CHRONIC MURINE	10 (50%)	14 (29%)	29 (62%)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(49)	(46)
ANGIECTASIS		1 (2%)	
HYPERPLASIA, LYMPHOID		2 (4%)	1 (2%)
#LYMPH NODE	(19)	(44)	(43)
HYPERPLASIA, LYMPHOID			1 (2%)
#MANDIBULAR L. NODE	(19)	(44)	(43)
HYPERPLASIA, LYMPHOID			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(47)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL(UNTR) 22-2176	LOW DOSE 22-2174	HIGH DOSE 22-2172
INFLAMMATION, NECROTIZING	1 (5%)		
INFLAMMATION, ACUTE FOCAL			1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU	1 (5%)		
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL		2 (4%)	
#PANCREAS	(19)	(49)	(43)
INFLAMMATION, ACUTE NECROTIZING	1 (5%)		
#STOMACH	(18)	(48)	(45)
INFLAMMATION, FOCAL		2 (4%)	
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, ACUTE FOCAL			1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#LARGE INTESTINE	(18)	(47)	(44)
PARASITISM		5 (11%)	4 (9%)
URINARY SYSTEM			
#KIDNEY	(20)	(48)	(46)
HYDRONEPHROSIS		1 (2%)	
GLOMERULONEPHRITIS, NOS			1 (2%)
INFLAMMATION, CHRONIC	1 (5%)		
NEPHROSIS, NOS			1 (2%)
INFARCT, FOCAL		2 (4%)	
AMYLOIDOSIS			1 (2%)
METAPLASIA, OSSEOUS		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(10)	(35)	(36)
CYSTIC FOLLICLES		3 (9%)	1 (3%)
INFLAMMATION, FOCAL	1 (10%)	1 (3%)	
INFLAMMATION, CHRONIC			2 (6%)
HYPERPLASIA, FOLLICULAR-CELL			2 (6%)
#THYROID FOLLICLE	(10)	(35)	(36)
HYPERPLASIA, FOCAL		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2176	LOW DOSE 22-2174	HIGH DOSE 22-2172
REPRODUCTIVE SYSTEM			
#UTERUS	(19)	(47)	(43)
DILATATION, NOS		1 (2%)	
CYST, NOS	1 (5%)	1 (2%)	1 (2%)
#UTERUS/ENDOMETRIUM	(19)	(47)	(43)
CYST, NOS	2 (11%)	2 (4%)	3 (7%)
HYPERPLASIA, NOS	1 (5%)	5 (11%)	10 (23%)
HYPERPLASIA, CYSTIC	10 (53%)	28 (60%)	21 (49%)
#OVARY	(17)	(32)	(27)
CYST, NOS	2 (12%)		3 (11%)
PAROVARIAN CYST			1 (4%)
HEMORRHAGIC CYST			1 (4%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(19)	(47)	(45)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	2 (4%)
#BRAIN	(19)	(47)	(45)
HEMORRHAGE			1 (2%)
PERIVASCULAR CUFFING		2 (4%)	
CORPORA AMYLACEA	5 (26%)	8 (17%)	19 (42%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(49)	(48)
PARASITISM			1 (2%)
BODY CAVITIES			
*MESENTERY	(20)	(49)	(48)
NECROSIS, FAT	2 (10%)	2 (4%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2176	LOW DOSE 22-2174	HIGH DOSE 22-2172
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(48)
HYPERPLASIA, LYMPHOID			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		2	
ANIMAL MISSING/NO NECROPSY		1	2
AUTO/NECROPSY/HISTO PERF			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

Review of the Bioassay of 2,4-Dimethyoxyaniline Hydrochloride*
for Carcinogenicity by the
Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens

August 31, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 2,4-Dimethyoxyaniline Hydrochloride for carcinogenicity.

Despite an increased incidence and positive trend in liver tumors in treated mice, the primary reviewer said that the evidence was not sufficiently convincing to conclude that 2,4-Dimethyoxyaniline Hydrochloride was carcinogenic in this species or in rats. He emphasized that the conclusion was based on the statistical analysis of the data. After a brief description of the experimental design, he noted the small number of control animals used. Because of the "disturbing" incidence of hepatocellular tumors in treated mice, the primary reviewer said that no statement could be made regarding the potential human risk of 2,4-Dimethyoxyaniline Hydrochloride.

The secondary reviewer agreed with the primary reviewer's critique. He noted the poor survival of female rats beyond 90 weeks. Despite the shortcoming, he said that he still considered the test to be adequate.

A motion was approved unanimously that the report on the bioassay of 2,4-Dimethyoxyaniline Hydrochloride be accepted as written.

Members present were:

Arnold Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

DHEW Publication No. (NIH) 79-1727